

Research Article

Evaluation of the Anti-Ulcer Activity of Ethanolic Extract of *Azadirachta indica* Flower

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ABSTRACT

The objective of present study is to evaluate the antiulcer activity of ethanolic extract of flower of *Azadirachta indica*. The cause of ulceration in patients is mainly due to hyper secretion of gastric acid and pepsin. Plant extracts and polyherbal formulations are some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of gastric ulcers. The anti-ulcer activity of ethanolic extract *Azadirachta indica* was investigated by pylorus ligation and indomethacin induced gastric ulcer in rats. The ethanolic extract of *Azadirachta indica* significantly reduced the ulcer produced by pylorus ligation. The ethanolic extract of *Azadirachta indica* at the dose of 200 and 400 mg/kg afforded 49.41% and 79.19% respectively, where as ranitidine 85.56 % against pylorus ligation induced ulcer. In Indomethacin induced gastric mucosal damage model, ethanolic extract of *Azadirachta indica* significantly reduced the incidence and severity of ulceration. The extract showed ulcer protection 50.81% and 75.97% in 200 mg/kg and 400 mg/kg doses respectively whereas the standard drug ranitidine exhibited 89.18% protection.

Keywords: Pylorus ligation, Aspirin, Indomethacin, Ranitidine, Anti-ulcer activity.

INTRODUCTION

Peptic ulcer is one of the most common gastrointestinal diseases. The term 'peptic ulcer' refers to an ulcer found in the lesser curvature of the antral end of the stomach or more rarely, in the lower end of the esophagus. It occurs in part of the gastrointestinal tract (GIT) where the gastric acid and pepsin is exposed to mucosa, i.e. the stomach and duodenum. It results probably due to an imbalance between the aggressive (acid, pepsin, bile and *H. pylori*) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, nitric oxide, innate resistance of the mucosal cells) factors^{1,2}.

Herbal medicines are fast emerging as an alternative treatment of ulcer possibly due to lower costs, availability, fewer adverse effects and perceived effectiveness. The number of antiulcer drugs such as H₂ receptor antagonists, proton pump inhibitors and cytoprotectants are available for ulcer treatment; however they exhibit systemic adverse effects. The antiulcerogenic activity of many plant products are reported due to an increase in mucosal defensive factors rather than decrease in the offensive factors. In market success of availability of medicinal products for treating ulcer are under limitation due to their adverse effects. Due to the

reported side effects of available antiulcer drugs, focused have been shifted towards natural products as the new sources of antiulcer agents. Various natural medicinal plants have been studied based on the tradition knowledge of their properties and confirmed to be useful in treating and managing ulcer³.

Neem has attracted worldwide prominence for its medicinal properties. Neem flower extract have been reported to exhibit immunomodulatory, anti-inflammatory, anti-malarial, antifungal, antibacterial, antioxidant, anti-carcinogenic properties⁴. Further, *Azadirachta indica* has known to possess array of phytoconstituents like flavonoids, alkaloids, coumarins, steroids, triterpenoids, et^{5,6}.

The present study was designed to investigate the anti-ulcer activity of ethanolic extract of *Azadirachta indica* flower.

MATERIALS AND METHODS

Collection and authentication of plant material

The fresh flowers of *Azadirachta indica* used for the present studies were collected from Palakkad, Kerala, in May 2013. It was authenticated by Mr. Prabhu Kumar, Scientist Kottakkal Aryavaidya Sala, and Kerala. The

flowers were dried under shade. The dried flowers were pulverized separately into coarse powder by a mechanical grinder and were used for extraction.

Preparation of Ethanolic Extract

The powdered material (150 g) was packed in Soxhlet extractor and extracted using ethanol as solvent. The temperature was maintained on an electric heating mantle with thermostat control. Appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extract was concentrated to syrupy consistency by using rotary flash evaporator. The concentrated extract was then air dried at room temperature, weighed and percentage yield was calculated and stored in air tight container in 2–8°C until used.

Experimental animals

Healthy Wistar albinos rats (150–200 g) of either sex were used for the experiment and were procured from the animal house of Srinivas College of Pharmacy, Mangalore. They were maintained under standard conditions (temperature 22 ± 2°C, relative humidity 60 ± 5% and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol (Approval No. SCP/CPCSEA/P12/F150/2012). All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health". The animals were acclimatized for at least one week before use.

Pharmacological activities

1. Acute oral toxicity
2. Antiulcer activity

Acute toxicity study^{7,8}

Acute toxicity study of ethanolic extract of the flowers of *Azadirachta indica* was determined in Wistar albino rats according to OECD guidelines No. 425. The animals were fasted overnight and the ethanolic extract 2000 mg/kg was administered orally. Animals were observed continuously for first 3 h and monitored for 14 days for mortality and general

behavior of animals, signs of discomfort and nervous manifestations.

Evaluation Antiulcer activity

Pylorus ligation induced Ulcer in rats⁹

The rats were divided into 5 groups of 6 each. The ulcer was induced in group II to group V by oral administration of aspirin (200 mg/kg) for 3 days and pylorus was ligated on the fourth day following 36 hour fasting. The group I was served as normal control. All the drug solutions were prepared using 0.1% Tween 80 and given 0.2 ml/200g of body weight, 1 hour prior to pylorus ligation. The different groups were assigned as described below,

Group I : Vehicle control (0.1% Tween 80, p.o.)

Group II : Toxic control, aspirin (200 mg/kg, p.o.)

Group III : Standard control, aspirin (200 mg/kg, p.o.) + Ranitidine (20 mg/kg, p.o.)

Group IV : Test low dose, aspirin (200 mg/kg, p.o.) + Ethanolic flower extract of *Azadirachta indica* (200 mg/kg, p.o.)

Group V : Test higher dose, aspirin (200 mg/kg, p.o.) + Ethanolic flower extract of *Azadirachta indica* (400 mg/kg, p.o.)

Under light ether anaesthesia, the abdomen was opened and the pylorus was ligated. At the end of 4 hours after the pyloric ligation, the animals were sacrificed by anesthetic ether. The stomach was removed, opened along with greater curvature and the ulcer index was determined. The gastric content was titrated against 0.1 N NaOH to find out the free acidity and total acidity.

Biochemical estimation

1. Estimation of ulcer index

The stomach was removed and opened. The number of ulcers per stomach was noted and severity of the ulcers scored microscopically. The score and ulcer index was calculated as follows,

Calculation of Ulcer Index

$$UI = UN + US + UP \times 10^{-1}$$

UI = Ulcer Index

UN = Average of number of ulcer per animal

US = Average of severity score

UP = Percentage of animal with ulcer

And percentage protection was observed by using the formula

$$\% \text{ protection} = \frac{(\text{Ulcer Index}) \text{ Control} - (\text{Ulcer Index}) \text{ Test}}{(\text{Ulcer Index}) \text{ Control}} \times 100$$

Estimation of acidity

The stomach was removed, opened along with greater curvature, gastric contents were centrifuged at 3000 rpm for 10 min. Volume was noted. The pH of gastric juice is recorded by pH meter.

Determination of free acidity and total acidity

One ml of gastric juice was diluted with 10 ml distilled water in 100 ml conical flask, added 2

to 3 drops of Topfer's reagent with 0.1N NaOH until all traces of red colour disappears and the solution turns to yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Then 2 to 3 drops of phenolphthalein was added and titration was continued until a definite red tinge reappears. Again the volume of alkali added was noted. This volume corresponds to total acidity.

Acidity was calculated by following formula

$$\text{Acidity} = \frac{\text{volume of NaOH} \times \text{Normality of NaOH}}{0.1} \times 100 (\text{meq/L}/100)$$

Indomethacin induced ulcer in rats¹⁰**Experimental Design**^{11,12}

Wistar rats weighing between 150 to 200 gm were randomly divided into 5 groups of 6 each. The ulcer was induced in group II to group V by oral administration of indomethacin (20 mg/kg). The group I was served as normal control. The different groups were assigned as described below.

Group I : Vehicle control (0.1% Tween 80, p.o.)

Group II : Toxic control, indomethacin (20 mg/kg, p.o.)

Group III: Standard control, indomethacin (20 mg/kg, p.o.) + Ranitidine (20 mg/kg, p.o.)

Group IV: Test low dose, indomethacin (20 mg/kg, p.o.) + Ethanolic flower extract of *Azadirachta indica* (200 mg/kg, p.o.)

Group V : Test higher dose, indomethacin (20 mg/kg, p.o.) + Ethanolic flower extract of *Azadirachta indica* (400 mg/kg, p.o.)

All the drug solutions were prepared using 0.1% Tween 80 and given 0.2 ml/200 g 10 minute prior to oral indomethacin administration. After 6 hours, rats were sacrificed and 2% v/v formal saline was injected into totally ligated stomach for overnight storage. Next day, stomach was opened along with greater curvature, washed with warmed water, and examined under a 3-fold magnifier. The length of the longest diameters of the lesions were measured and summated to give a total lesion score (in mm) for each animal, the mean count for each group and ulcer index was observed. Protection of the lesion induction was expressed as percentage value.

Statistical analysis

Results of biochemical estimation were reported as mean \pm S.E.M. The total variation present in a data was analyzed by one way analysis of variance (ANOVA). P value less

than 0.05 was considered as statistically significant.

RESULTS**Evaluation of anti-ulcer activity of ethanolic extract *azadirachta indica* flower**
Anti-ulcer effect of ethanolic extract against pylorus ligation induced ulcers in rats

Toxic control (aspirin) groups showed highly significant ($p < 0.001$) increase in free acidity, total acidity and in volume of gastric juice and decrease in pH levels compared to normal control group.

Standard ranitidine treated group showed extremely significant ($p < 0.001$) decrease in free acidity, total acidity and increase in volume of gastric juice and pH levels compared to toxic control group.

The animals pre-treated with ethanolic extracts of *Azadirachta indica* flower 200 mg/kg showed moderately significant ($p < 0.01$) decrease in volume of gastric juice, total acidity and ulcer index level, where as less significant ($p < 0.05$) increase pH and decrease in free acidity.

The animals pre-treated with Ethanolic extract of *Azadirachta indica* 400 mg/kg showed highly significant ($p < 0.001$) decrease in volume of gastric juice, total acidity and ulcer index level, where as moderately significant ($p < 0.01$) increase in pH and decrease in free acidity level.

The ethanolic extract of *Azadirachta indica* reduced the ulcer produced by pylorus ligation. The ethanolic extract of *Azadirachta indica* at the dose of 200 and 400 mg/kg afforded 49.41% and 79.19% respectively, where as ranitidine 85.56 % against pylorus ligation induced ulcer.

The ulcer protection action at 400 mg/kg of ethanolic extract of *Azadirachta indica* was found to be closer to the standard drug Ranitidine. (Table 1)

Anti-ulcer effect of ethanolic extracts of *Azadirachta indica* flower against indomethacin induced ulcer in rats

In Indomethacin induced gastric mucosal damage model, ethanolic extract of *Azadirachta indica* significantly reduced the incidence and severity of ulceration. The extract showed ulcer protection 50.81% and 75.97% in 200 mg/kg and 400 mg/kg doses respectively whereas the standard drug ranitidine exhibited 89.18% protection (Table 2).

DISCUSSION

The ethanolic extract of *Azadirachta indica* containing alkaloids, carbohydrates, glycoside, tannins, proteins, and flavonoids. The ethanolic extract of *Azadirachta indica* flower has significantly decreased the secretion of gastric factors like volume of gastric juice, free acidity and total acidity¹³. These results suggested that ethanolic extract of *Azadirachta indica* possesses anti-secretory effect as well as reduced ulcer index¹⁴. The ethanolic extract of *Azadirachta indica* reduced the ulcer production at the dose of 200 and 400 mg/kg, afforded 49.41% and 79.19% respectively, where as ranitidine 85.56% protection against pylorus ligation induced ulcer. Indomethacin induces gastric lesion in rats by inhibition of gastric cyclo- oxygenase resulting in less formation of endogenous

prostaglandin, also inhibits mucosal blood flow as well as gastro duodenal bicarbonate secretion¹⁵. The results of the present study revealed that the presence of various phytoconstituents in the ethanolic extract of *Azadirachta indica* flower might be responsible for gastric ulcer protection against pylorus ligation and indomethacin induced ulcers by both reduction in gastric acid secretion and gastric cytoprotection.

CONCLUSION

The ethanolic extract of *Azadirachta indica* flower in pylorus ligation and indomethacin induced gastric ulcer displayed appreciable gastro protective activity as demonstrated by significant decrease in ulcer index and increased percent protection in both models. From the above data it can be concluded that the ethanolic extract of *Azadirachta indica* flower exhibited a significant, dose dependent anti-ulcer activity against both pylorus ligation and indomethacin induced gastric ulcer in rats. The various phytoconstituents present in the extract might contribute to the anti ulcer activity of the plant.

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Table 1: Values of Biochemical Parameters

Group	Treatment	Volume of gastric juice(ml)	pH	Free acidity meq/ltr	Total acidity meq/ltr	Ulcer index	% protection
Normal	0.1% Tween 80	1.14±0.19	4.60±0.019	9.74±0.35	21.34±0.88	–	100
Toxic control	Aspirin 200 mg/kg	4.26±0.13#	1.87±0.028#	40.39±2.58#	110.2±9.95#	15.38±0.13#	–
Standard	Ranitidine 20 mg/kg	2.11±0.06***	4.41±0.048** *	12.81±0.79***	25.66±0.35***	2.22±0.06***	85.56
Low dose	<i>Azadirachta indica</i> Extract 200 mg/kg	2.63±0.33**	3.44±0.051*	25.64±4.83*	68.44±2.56**	7.78±0.04**	49.41
High dose	<i>Azadirachta indica</i> Extract 400 mg/kg	2.48±0.16***	3.89±0.02**	21.63±5.00**	60.19±3.45***	3.20±0.05***	79.19

All the values are mean ±SEM, n=6, *p<0.05, **p<0.01, ***p<0.001, One way ANOVA followed by Dunnett's test compared to toxic control. Toxic control#p<0.001vs normal control

Table 2: Effect of ethanolic extracts of *Azadirachta indica* flower on indomethacin induced ulcer in rats

Group	Treatment	Dose	Ulcer index	% Protection
Normal	0.1% Tween 80	—	—	100
Toxic control	Indomethacin	20 mg/kg	14.15±0.61	—
Standard	Ranitidine	20 mg/kg	1.53±0.83***	89.18%
Low dose	<i>Azadirachta indica</i> extract	200 mg/kg	6.96±0.14**	50.81%
High dose	<i>Azadirachta indica</i> extract	400 mg/kg	3.40±0.38***	75.97%

All the values are mean ±SEM, n=6, *p<0.05, **p<0.01, ***p<0.001, One way ANOVA followed by Dunnett's test compared to toxic control. Toxic control#p<0.001 vs. normal control

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